

Animal Care and Ethics Committee

Guideline for euthanasia of embryonic, foetal and early neonate animals

INTRODUCTION

Euthanasia is a method of ending an individual animal’s life in a manner that minimises pain and distress. As a general principle, animals are killed by the most humane method for that species. However, the reproducibility and variability of scientific data collected from animals in an experiment can be affected by the way in which animals are killed. Any alternative method of euthanasia can only be considered if it is established to the satisfaction of the UNSW Animal Care and Ethics Committee that the effects of the euthanasia method on data would compromise the scientific validity of a research proposal.¹²

EUTHANASIA OF EMBRYONIC, FOETAL AND EARLY NEONATE ANIMALS

The method of euthanasia must be appropriate for the age of the animal. Choosing an appropriate euthanasia method for animals that are immature (early neonates) or at a very early stage of development (embryonic or foetal stage) requires special consideration, taking into account two key factors¹²:

1. Resistance to hypoxia and hypercapnia due to an underdeveloped respiratory system
2. Slower drug metabolism

It is also important to note the developmental differences between rodents with altricial and precocial young. Altricial neonates (mice and rats) are born or hatched helpless requiring extensive parental care while precocial species (guinea pigs) are those where the young are born relatively mature and mobile immediately after birth or hatching. Generally, altricial neonates are resistant to hypoxia while precocial neonates are susceptible to hypoxia and should be given the same ethical considerations that apply to a fully mature animal.

Mammalian foetuses are unconscious while inside the uterus due to a combination of factors, e.g. low oxygen tension and hormonal influences in the uterus causing suppression of consciousness so they cannot consciously experience feelings such as breathlessness or pain during euthanasia. Consequently, they also ‘cannot’ suffer while dying in utero after the death of the dam ².

However, foetuses removed from the amniotic sac after euthanasing the dam can potentially breathe and perceive pain (mouse, rat and hamster greater than embryonic day E15; guinea pigs greater than E35) ^{16, 19} consequently should be euthanased using the recommended procedures for the species.

As a single euthanasia method may not be as effective as in adult animals, it is recommended that two acceptable methods for the species should be combined. Death should be confirmed using similar clinical signs as described for adult animals. ¹²

Because of species-specific differences in development during gestation, the choice of euthanasia of embryos and neonates should be based on the best available data, with attention to assuring, as best as possible, that conscious suffering does not occur. ²

REPORTING IN NSW

In NSW, the annual reporting form for animal use [NSW DPI Form L: Animal Use Statistics](#) requires reporting of immature stages of development to include embryonic, foetal and larval forms once the development of that form has progressed beyond half the gestation or incubation period for the species, or it becomes capable of independent feeding, whichever is sooner.

Species	Stage of reporting
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Mammals	From half-gestation onwards
Birds	From half-incubation onwards
Reptiles	From half-incubation onwards
Amphibians	Fully metamorphosed juveniles and older
Fish	Fully metamorphosed juveniles and older

Although the use of immature animals less than halfway of gestation does not require reporting to the NSW DPI, their use still requires approval from the Animal Ethics Committee.

EUTHANASIA OF MICE AND RATS

Mice and Rats Foetuses: Choose a procedure that will ensure foetal cerebral anoxia and minimal disturbance to the uterus to minimise foetal arousal. ³ Mice and rat foetuses are unconscious in utero and does not respond to hypoxia.

DEVELOPMENTAL STAGE/AGE	ACCEPTABLE	ACCEPTABLE WITH CONDITIONS
Foetus not required for research	<ul style="list-style-type: none"> Dam euthanased using CO2 then cervical dislocation ³ Leave pups in-utero ¹¹ 	
Foetus required for research (up to Embryonic Day 15) ³ .	<ul style="list-style-type: none"> Dam euthanased using CO2 or inhaled anaesthetic then cervical dislocation 	
Foetus required for research (over Embryonic day 15) ^{2,3,5,7}	<ul style="list-style-type: none"> Injectable barbiturates Injectable barbiturates + local anaesthetics/ anticonvulsants/ dissociative agents with α-adrenergic receptor agonist or benzodiazepines 	<ul style="list-style-type: none"> Hypothermia followed by secondary euthanasia method Decapitation (use sharp surgical scissor/ blade) Cervical Dislocation (pinching and disrupting the spinal cord in the high cervical region)

Mice and Rats Early neonates (up to 10 days of Age): Rat and mouse pups are born neurologically immature as compared to humans. Their afferent pain pathways are not well developed until after postnatal day 5 to 7, with cortical development occurring later. ²

DEVELOPMENTAL STAGE/AGE	ACCEPTABLE	ACCEPTABLE WITH CONDITIONS
Foetus not required for research	<ul style="list-style-type: none"> IP injection with barbiturates, anaesthetics Cervical dislocation 	<ul style="list-style-type: none"> Prolonged Hypothermia ¹³ - chilling of pups on crushed ice for 1-2 minutes or until cessation of movement Decapitation ^{3,7} Cervical Dislocation ³ Inhalation anaesthetics – prolonged exposure required CO2 ¹⁰ – prolonged exposure required*

* CO2 euthanasia of neonatal mice: may take up to 50 minutes to die from CO2 exposure on day of birth and differed between inbred and outbred strain. ¹⁴

CO2 euthanasia of neonatal rats: time to death varied inversely with age, decreasing steadily by 3 minutes for every 1 day increase in age from birth with 100% of neonates euthanased after 5 min of CO2 exposure at 10 days of age. ¹⁵

Suggested refinements for euthanasia methods

Euthanasia Method	Refinement
Hypothermia	<ul style="list-style-type: none"> Do not expose directly to ice. Place pups on gauze or thick paper towel to prevent pain from direct exposure of skin to cold temperature Require secondary form of euthanasia to avoid the possibility of recovery, e.g. decapitation or cervical dislocation
Decapitation	<ul style="list-style-type: none"> Prior sedation by hypothermia Use sharp surgical instruments (scissors or scalpels) Competency
Inhalation Anaesthetics or CO2	<ul style="list-style-type: none"> Require secondary form of euthanasia e.g., decapitation or cervical dislocation to avoid the possibility of recovery

Mice and Rats Early neonates (Day 7 to weaning): Follow recommendations for adults.

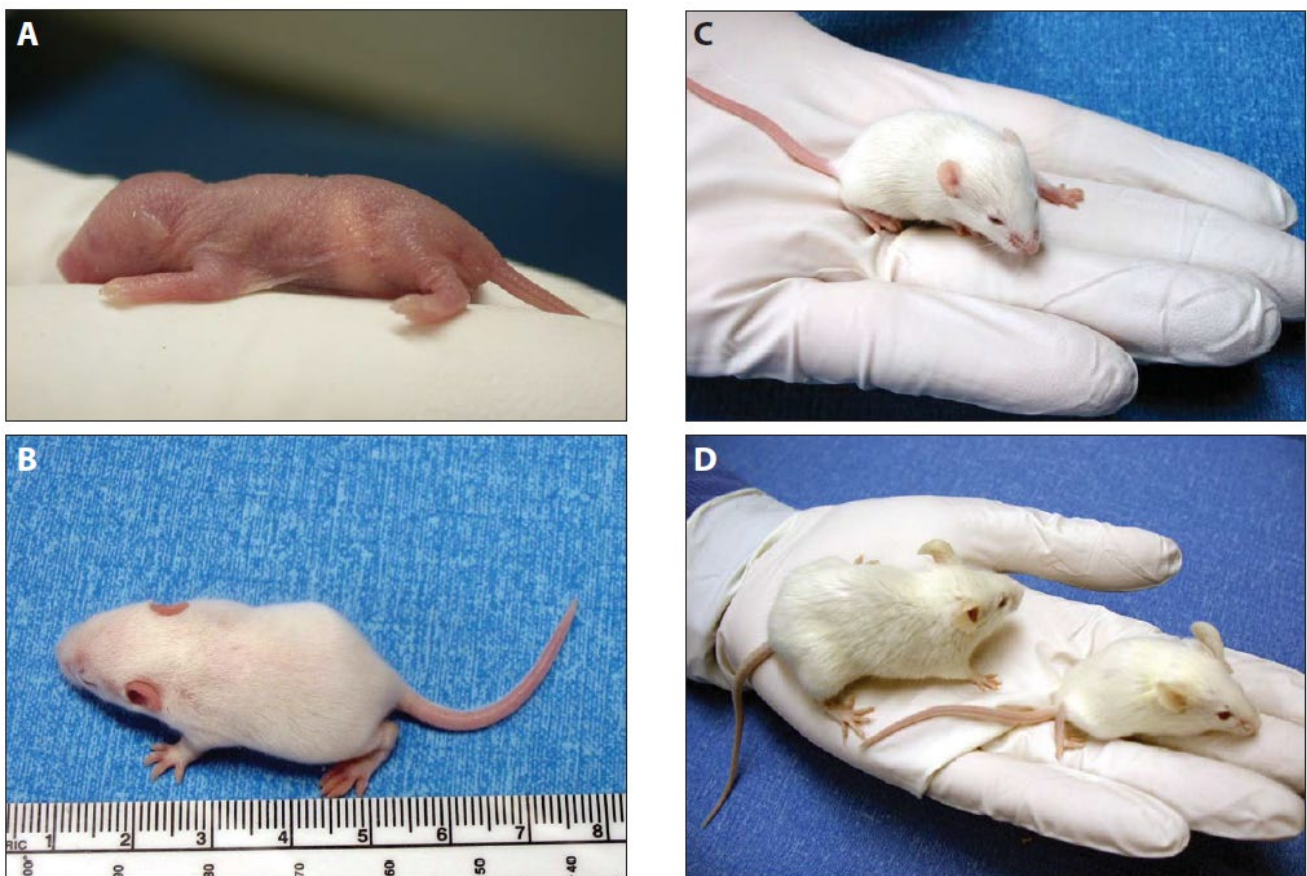


Figure 2. Representative animals at ages mentioned in Table 3. Animals are BALB/cJ mice at (A) 3 days old, (B) 10 days old, (C) 14 days old, and (D) 21 days old (right), and adult (left).

Figure 1. Image sourced from Prichett et al. 2005

EUTHANASIA OF GUINEA PIGS

Guinea pigs Foetuses: remain insentient and unconscious until 75% to 80% of gestational development and remain unconscious until after birth due to chemical inhibitors (e.g, adenosine, allopregnanolone, pregnanolone, prostaglandin D2, placental peptide neuroinhibitor) and hypoxic inhibition of cerebrocortical activity. ²

Guinea pigs Neonates: precocial species and should be treated as adults due to their advanced development. ²

DEVELOPMENTAL STAGE/AGE	ACCEPTABLE	ACCEPTABLE WITH CONDITIONS
Foetus required for research (< embryonic day 35)	<ul style="list-style-type: none"> Dam euthanased using CO2 or inhaled anaesthetic then cervical dislocation of foetus 	
Foetus required for research (> embryonic day 35) ²	<ul style="list-style-type: none"> Injectable barbiturates Injectable barbiturates + local anaesthetics/ anticonvulsants/ dissociative agents with α-adrenergic receptor agonist or benzodiazepines 	<ul style="list-style-type: none"> Decapitation (use sharp surgical scissor/ blade/other decapitation tool) Cervical Dislocation (pinching and disrupting the spinal cord in the high cervical region)

EUTHANASIA OF BIRDS

Bird embryos **halfway through gestation** (when the neural tube has developed into a functional brain) are capable of perceiving pain and must be destroyed humanely. ⁷

However, there are scientific evidence that chick embryo's ability to experience pain starts to develop at **Day 7 incubation** so steps should be taken to avoid suffering of the embryo starting from this developmental stage. ¹

DEVELOPMENTAL STAGE/AGE	ACCEPTABLE	ACCEPTABLE WITH CONDITIONS
Bird Embryo	<ul style="list-style-type: none"> Injectable barbiturates and anaesthetics chorioallantoic vascular system or intracoelomic (intraabdominal) ¹ Decapitation Cooling (<4°C for 4 hours) then decapitation or freezing (<80% incubation) ^{2,7} Disruption of the membranes and maceration (specialist apparatus) ^{2,7} Prolonged exposure to CO2 (> 20 minutes) ^{2,4} Inhaled anaesthetics administered through the air cell at the large end of the egg. ² 	
Newly Hatched (up to 72 hours old)	<ul style="list-style-type: none"> Sodium pentobarbitone i.p. or i.v. with local anaesthetic (all ages) ⁵ Inhalant anaesthetics followed by another method to ensure death ⁵ Concussion (hard blow to the head) followed by destruction of the brain (day old) ⁷ Maceration (specialist apparatus) (up to 72 hrs old) ^{2,7} 	<ul style="list-style-type: none"> Decapitation in unconscious birds (use sharp surgical scissor/ blade/other decapitation tool) ⁷ Cervical Dislocation (day old, small number of birds to avoid human error due to tiredness) ^{7,9} Prolonged exposure to CO2 (up to 72 hrs old) ^{7,8,9} Maceration (specialist apparatus) (up to 72 hrs old) ^{2,7}

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